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Produktinformation

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Firefly Luciferase-eGFP Lentivirus (EF1A Promoter, Blasticidin) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. These viruses transduce constitutively expressing firefly luciferase and eGFP (enhanced green fluorescent protein) under an EF1a promoter. The lentiviruses also transduce a blasticidin selection marker (Figure 1).

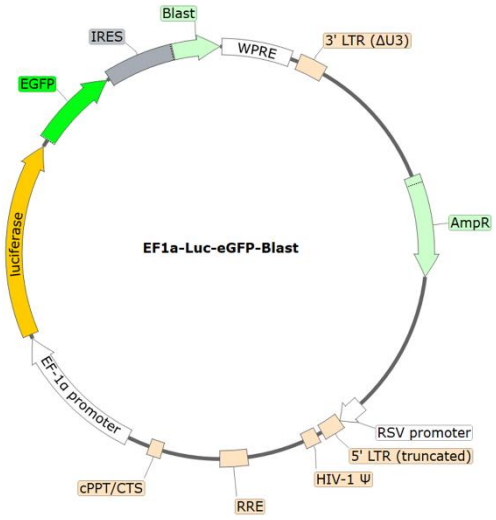

Figure 1. Schematic of the lenti-vector used to generate the Firefly Luciferase-eGFP Lentivirus (EF1A Promoter, Blasticidin).

Background

Firefly luciferase has been used as a sensitive reporter to study a wide range of biological responses. It was first cloned from the North American *Photinus pyralis* and catalyzes the oxidation of D-luciferin, in the presence of ATP and magnesium, emitting yellow light. This reaction has a high quantum yield and both luciferase and luciferin have low toxicity. These characteristics contributed to Firefly luciferase becoming a commonly used tool in biology. GFP (green fluorescent protein) presents green fluorescence, and it was first identified in *Aequorea Victoria*. It has become widely used in cell biology to monitor gene expression, protein localization, and protein-protein interactions. Its popularity prompted the development of mutant variants, such as the eGFP (enhanced GFP). eGFP has a higher intensity emission compared to the GFP molecule. The use of firefly luciferase as reporter system allows for easy readouts, and high throughput screening, while the presence of GFP allows for easy visualization and cell sorting.

Application

- Use as transduction positive control.
- Optimize transduction assays.
- Generate firefly luciferase- and eGFP-expressing cell pools or stable cell lines following blasticidin selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the protocol described in the "Validation Data" section. Media and reagents used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated assay plates	

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for the Proposed Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using the Firefly Luciferase-eGFP Lentivirus (EF1A Promoter, Blasticidin). The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target gene with the appropriate antibiotic prior to carrying out the reporter assays.

Day 1:

1. Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into a clear-bottom 96-well microplate in 100 µl of Thaw Medium 1 (#60187).

2. Incubate the cells at 37°C with 5% CO_2 overnight.

Day 2:

1. Add 1 µl of Firefly Luciferase-eGFP Lentivirus (EF1A Promoter, Blasticidin) into each well.

2. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix.

3. Incubate the plate at 37°C with 5% CO_2 overnight.

   Note: Alternatively, cell seeding and transduction can be performed at the same time.

Day 3:

1. Remove the medium containing the lentivirus from the wells.

2. Add 100 µl of fresh Thaw Medium 1 to each well.

   Note: If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

Day 4-5:

1. Approximately 48-72 hours after transduction, ONE-Step™ Luciferase reagent (#60690) was added to cells to measure the luciferase activity. The expression of eGFP in the target cells can be examined under a fluorescence microscope or by flow cytometry.

Notes

To generate a firefly luciferase-eGFP stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of blasticidin (as pre-determined from a killing curve, https://bpsbioscience.com/kill-curve-protocol), for antibiotic selection of transduced cells followed by clonal selection.

Figures and Validation Data

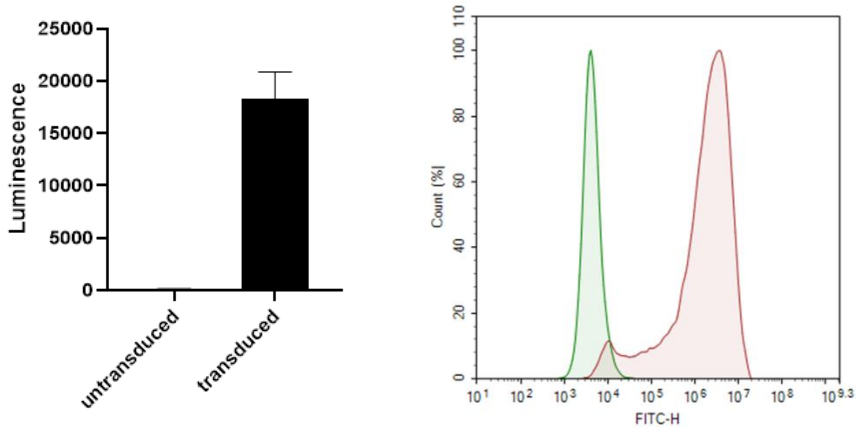

Figure 2. Luciferase activity and eGFP expression in HEK293 cells transduced with Firefly Luciferase-eGFP Lentivirus (EF1A Promoter, Blasticidin).
Left panel: 10,000 HEK293 cells were infected with 0.5 µl of Firefly Luciferase-eGFP Lentivirus (EF1A Promoter, Blasticidin). 66 hours post-transduction ONEStep™ Luciferase reagent (#60690) was added to the cells to measure luciferase activity. Non-transduced cells were used as control. Right panel: 30,000 HEK293 cells were infected with 1.5 µl of Firefly Luciferase-eGFP Lentivirus (EF1A Promoter, Blasticidin). 66 hours post-transduction, the expression of eGFP was analyzed by flow cytometry (red). Non-transduced cells were used as control (green). The y axis represents the % of cells while the x axis indicates the fluorophore intensity.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Firefly Luciferase-eGFP Lentivirus (G418 or Puromycin)	79980	500 µl x 2
Firefly Luciferase Lentivirus (EF1A Promoter/ Geneticin, Hygromycin, Puromycin or Blasticidin)	78741	500 µl x 2
Enhanced GFP Lentivirus (G418, Hygromycin and Puromycin)	78639	500 µl x 2
Firefly Luciferase Lentivirus (G418, Hygromycin and Puromycin)	79692	500 µl x 2
Renilla Luciferase Lentivirus (G418 or Puromycin)	79565	500 µl x 2
Firefly Luciferase Lentivirus (Ubc Promoter)	79880	500 µl x 2

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